VOSSIUS & PARTNER

Vossius & Partner POB 86 07 67 81634 Munich Germany

European Patent Office
Erhardtstr. 27

80298 M Ü N C H E N

PATENTANWÄLTE
EUROPEAN PATENT ATTORNEYS
EUROPEAN TRADEMARK ATTORNEYS

Dr. VOLKER VOSSIUS, Dipl.-Chem.
(bis 1992; danach in anderer Kanzlei)
Dr. PAUL TAUCHNER, Dipl.-Chem.
Dr. DIETER HEUNEMANN, Dipl.-Phys.
Dr. PETER A. RAUH, Dipl.-Chem.
Dr. GERHARD HERMANN, Dipl.-Phys.
JOSEF SCHMIDT, Dipl.-Ing.
Dr. HANS-RAINER JAENICHEN, Dipl.-Biol.
Dr. ALEXA VON UEXKÜLL, M.Sc.
Dr. RUDOLF WEINBERGER, Dipl.-Chem.
AXEL STELLBRINK, Dipl.-Ing.
Dr. JOACHIM WACHENFELD, (Biol.)
Dr. FRIEDERIKE STOLZENBURG, Dipl.-Biol.
RAINER VIKTOR, Dipl.-Ing.
Dr. NATALIA BERRYMAN, Dipl.-Chem.
Dr. JÜRGEN MEIER, Dipl.-Biol.

EUROPEAN PATENT ATTORNEYS

Dr. RENATE BARTH, Dipl.-Chem.
Dr. URSULA ENGLBRECHT, Dipl.-Chem.
Dr. PETER EINMAYR, Dipl.-Chem.

RECHTSANWÄLTE

HELGA TREMMEL
Dr. JOHANN PITZ
BARBARA GUGGENMOS, Dipl.-Chem.
Dr. THURE SCHUBERT
SIMONE SCHÄFER
JENNIFER CLAYTON-CHEN

Partnerschaftsregister Amtsgericht München PR 89

PCT/EP2004/005936
B.R.A.I.N. AG
Our Ref.: G 3126 PCT

November 2, 2004
WA/DU/OOV

This is in response to the Written Opinion pursuant to Rule 43bis.1 PCT issued by the European Patent Office with the International Search and Trust Authority on September 2, 2004.

Please find enclosed a new set of claims 1 to 20, in triplicate.

1. AMENDMENTS TO THE CLAIMS

1.1 New claim 1 is based on original claim 1. Technical features of original claim 4 have been introduced into item (c) of new claim 1. The amendment finds support in the description as originally filed on page 10, lines 3 and 4.

1.2 New claims 2 to 20 correspond to originally filed claims 2, 3 and 5 to 21 with adapted back references.

VISITING ADDRESS:
Siebertstrasse 3
81675 Munich
Germany

POSTAL ADDRESS:
POB 86 07 67
81634 Munich
Germany

TEL.: +49-89-41 30 40
FAX: +49-89-41 30 41 11
+49-89-41 30 44 00 (Trademarks)
EMAIL: info@vossiusandpartner.com
HOMEPAGE: www.vossiusandpartner.com

2. INVENTIVE STEP (ARTICLE 33(3) PCT)

In section 2.1 of the Written Opinion the Examiner considers document D2 to represent the most relevant state of the art for the present invention. The document describes a sulfolobus expression vector which is designated as pPKMSW72. The vector is derived from the vector pKMSD and contains the sulfolobus beta-galactosidase gene lacS as reporter gene.

Furthermore, the Examiner has referred to document D3 which describes a further vector (pMJ02a) which is very similar to the pKMSW72 vector and also comprises an open reading frame (ORF) of lacS but under the control of the heat-inducible promoter tf55.

At the day of priority of the present application is was known to the person skilled in the art from the diploma thesis of M. Jonuscheit (TU Darmstadt, 2002) that transformation of Sulfolobus with the two vectors, pKMSW72 and pMJ02a, does not result in a stable transformation of the bacteria. The according results of the diploma thesis are summarized in the enclosed figures 1 and 2 and table 1.

In view of said state of the art the objective technical problem underlying the present invention was to provide means and methods for a stable transformation of Sulfolobus.

Said technical problem has been solved by the provision of a Sulfolobus expression vector according to new claim 1. The essential technical feature by which the Sulfolobus expression vector of the present invention can be distinguished from Sulfolobus vectors known in the art is that the present vector comprises one or more selectable marker genes which encode an essential protein of Sulfolobus. As generally accepted by the person skilled in the art, the term "genes which encode an essential protein" defines a group of genes the deletion of one of such genes is lethal to the organism; see e.g. an according definition in the glossary or Levin, Genes V, Oxford University Press, 1994.

It has been surprisingly found that only the inclusion of such gene provides a genetically stable and high-efficient cloning and expression system for Sulfolobus which has been described in the present application to be the technical problem underlying the present invention; see page 2, last paragraph. The inclusion of such gene into a Sulfolobus expression vector has been neither described nor suggested in the state of the art.

Accordingly, new claim 1 is inventive over document D2 or a combination of document D2 with D3 or D4. The same holds true for claims 2 to 20 which directly or indirectly depend on new claim 1.

3. REQUESTS

It is requested that in view of the amended set of claims and the above-provided arguments objections under Article 33(3) PCT are withdrawn.

In case a Chapter II may be requested for the present application it is further requested that a positive IPER for the present application be issued. Otherwise, it is requested that the above comments are communicated to all designated offices in accordance with the decision of the PCT assembly mentioned under item 292(d) in the applicant's guide for PCT applications (version of January 1, 2004).

Dr. Joachim Wachenfeld
European Patent Attorney

Enclosure:
New set of claims 1 to 20

CLAIMS

1. A sulfolobus expression vector comprising:
   (a) a sulfolobus origin of replication;
   (b) the genes encoding the structural proteins and the site-specific integrase of SSV1, SSV2 or pSSVx, operatively linked to expression control sequences and a packaging signal;
   (c) one or more selectable marker gene(s) encoding an essential protein of sulfolobus, operatively linked to sulfolobus expression control sequences; and
   (d) a sulfolobus promoter followed 3' by a restriction enzyme recognition site or a multiple cloning site for insertion of a gene of interest and optionally a 3' regulatory element.

2. The expression vector of claim 1, wherein the origin of replication of (a) is selected from the group consisting of SSV1, SSV2, pSSVx and pRN plasmids.

3. The expression vector of claim 1 or 2, wherein the vector contains the complete genome of SSV1, thereby providing said origin of replication, said packaging signal and said genes encoding the structural proteins and the integrase of SSV1.

4. The expression vector of claim 3, wherein the essential gene is a gene of the de novo nucleotide anabolism, a gene of the aminoacid biosynthesis or a gene conferring antibiotic resistance

5. The expression vector of anyone of claims 1 to 4, wherein the vector contains orotidine-5'-monophosphatase pyrophosphorlyase and orotidine-5'-monophosphatase decarboxylase as selectable marker genes.

6. The expression vector of any one of claims 1 to 5, wherein the vector contains 3' to the translation initiation site of the promoter for the expression of the gene of interest additional nucleic acid sequences so that the expressed protein has an N-terminal extension.

7. The expression vector of claim 6, wherein the N-terminal extension is
   (a) a signal sequence directing the secretion of the expressed protein;
   (b) a tag for purification; or
   (c) a tag for specific detection.

8. The expression vector of any one of claims 1 to 7, wherein the promoter for the expression of the gene of interest is a constitutive promoter selected from the group consisting of genes involved in central metabolisms and information processing including the promoters of the ribosomal subunits 16S, 23S rRNA or the promoters of polymerases, transcription, replication or translation factors.

9. The expression vector of any one of claims 1 to 8, wherein the promoter for the expression of the gene of interest is an inducible promoter.

10. The expression vector of claim 9, wherein the inducible promoter is selected from the group consisting of (a) heat inducible promoters Tf55alpha, TF55beta, TF55gamma, hsp20, htrA, (b) cold inducible promoters TF55gamma and (c) promoters inducible by a carbon source.

11. The expression vector of any one of claims 1 to 10, wherein the vector contains an additional expression cassette for a reporter protein, selected from the group consisting of ß-galactosidase, luciferase, green fluorescent protein and variants thereof.

12. A shuttle vector comprising the sequences of the expression vector of any one of claims 1 to 11 and additional sequences for propagation and selection in E. coli, wherein the additional sequences comprise
    (a) an E.coli ori of replication; and
    (b) a marker for selection in E.coli.

13. The shuttle vector of claim 12, wherein the marker of selection is selected from the group consisting of ampicillin, kanamycin, chloramphenicol, tetracyclin, hygromycin, neomycin or methotrexate.

14. A host cell transformed with the expression vector of any one of claims 1 to 13, wherein the host cell is E. coli or sulfolobus.

15. The host cell of claim 14, wherein the transformed expression vector provides a gene encoding an essential protein.

16. The host cell of claim 14, wherein the host is deficient in expressing a fully functional version of said essential gene provided by the expression vector.

17. A method of producing a polypeptide comprising culturing the host cell of any one of claims 14 to 17 under suitable conditions and isolating said (poly)peptide from the cells or the cell culture supernatant.

18. A method of generating infectious recombinant subviral particles composed of the structural proteins of SSV1 and/or SSV2, having packaged the DNA of the expression vector of any one of claims 1 to 13, wherein the method has the steps of
    (a) introducing the DNA of the expression vector and the DNA of SSV1 or SSV2 into a host cells;
    (b) incubating the cells for time and under conditions sufficient to allow replication of SSV1 or SSV2 and spreading in the cell culture;
    (c) harvesting the cell culture supernatant or the host cells.

19. Use of the vector of any one of claims 1 to 13 for gene silencing by expression of RNAi or antisense RNA, wherein the vector contains a Sulfolobus promoter for transcription of a gene or parts of a gene either in antisense or sense orientation or in both orientations.

20. A kit comprising
    (a) the vector of any one of claims 1 to 13,
    (b) the host cell of any one of claim 14 to 16, and/or
    (c) a host cell deficient in the expression of the essential protein of the vector of (a).

    in one or more containers.